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In this issue:

PRE ANALYTICAL ERRORS

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PRE ANALYTICAL ERROS

Introduction

A laboratory error is defined as any defect that occurs during the entire testing process, from ordering tests to reporting results. Any error during the laboratory testing process can affect patient care, including delay in reporting, unnecessary redraws, misdiagnosis, and improper treatment. Although errors can arise at any of the three stages, namely, Pre analytical, analytical, and post analytical phase, studies show that the pre-analytical phase accounts for 46% to 68.2% of errors observed during the total testing process due to its complexity, that is, due to the presence of many steps that occur both before and after the specimen reaches the laboratory. To reduce these errors, it is important to understand where they come from—especially those that happen before the actual testing begins. It is also necessary to know how much these errors can affect patient results. Setting acceptable limits for such variations, based on statistical and biological factors, helps in developing a good quality control program that minimizes their impact on patient care.

Sources of Preanalytical phases and how to avoid them are discussed below.

1. Biological Rhythms and Laboratory Results

Certain substances in our body show predictable time-based changes, known as **biological rhythms**, which reflect normal body functions and needs. Different analytes follow different timing patterns—some vary within hours, while others change over days or even months. Understanding these rhythms helps ensure accurate interpretation of laboratory test results.

Biological rhythms are generally classified into **circadian**, **ultradian**, and **infradian** types:

- **Circadian Rhythm (24-hour cycle):**
  This is our body's internal clock. In healthy individuals, hormones like **ACTH** and **cortisol** are higher in the morning (around 4:00–12:00 a.m.) and lower in the evening and night.
- **Ultradian Rhythm (less than 24 hours):**
  These are shorter cycles that occur several times a day. Some

substances, such as **testosterone**, are released in pulses throughout the day and typically peak between 10:00 a.m. and 5:00 p.m.

- **Infradian Rhythm (more than 24 hours):**
  These are longer cycles, such as the **menstrual cycle**, which lasts around 28–32 days. Hormones like **FSH**, **LH**, and **ovarian hormones** vary during different phases of this cycle. For example, FSH and LH show daytime peaks about 15–18% higher than nighttime levels during the early follicular phase. Therefore, fertility hormone test results must always be interpreted according to both the **phase of the menstrual cycle** and the **time of day** when the sample was taken.

1. Patient Preparation

Proper patient preparation is essential to ensure that laboratory test results are accurate and reliable. Several factors—such as **diet, exercise, medications, posture**, and **time of sample collection**—can significantly influence test outcomes.

a) Influence of Diet

- Eating a meal increases **blood glucose** and **triglyceride** levels, so glucose tests should be performed **after overnight fasting**.
- To ensure accurate measurement of **catecholamines**, patients should avoid foods and drinks containing **chocolate, caffeine, coffee, and tea** for several days before collecting 24-hour urine or plasma samples.

b) **Influence of Exercise**

- Exercise increases **catecholamines**, **β-endorphin**, **cortisol**, **glucagon**, **growth hormone (GH)**, and **prolactin**.
- **Strenuous exercise** also raises levels of **creatinine kinase**, **creatinine**, and **aspartate aminotransferase (AST)**.

c) Influence of Posture

- Moving from lying down to standing causes fluid to shift to the legs, reducing **plasma volume by about 14%** after 30 minutes.
- This leads to higher concentrations of **proteins** such as **albumin**, **α2-macroglobulin**, **transferrin**, and **total protein**, as well as **protein-bound analytes** like **calcium**.

d) Therapeutic Drug Monitoring (TDM)

When monitoring drug levels, the **timing of sample collection** is very important.

- The **trough level** is the lowest drug concentration in the blood, measured **just before the next dose**.
- After taking the medication, the level rises to a **peak**, then gradually falls as the drug is metabolized and eliminated.
- Collecting the specimen **right before the next dose** ensures that the trough concentration is measured accurately.

2. Whole Blood, Plasma, and Serum Specimens

In clinical laboratory testing, **whole blood, plasma, and serum** are all important specimen types. Each has unique properties that determine its **suitability for specific laboratory tests**.

a) Whole Blood

Whole blood is used not only for examining cellular components but also for testing substances that are mainly found inside blood cells.

- **Erythrocytes (red blood cells)** can act as easily accessible tissue samples and often give a better picture of how certain analytes are distributed in the body. Examples of such analytes include **vitamins, trace elements, and certain drugs**.
- Whole blood is also commonly used for **point-of-care testing**, such as bedside or rapid diagnostic tests. These samples are usually collected by **skin puncture**, producing what is commonly known as **capillary blood**.

b) Plasma and Serum

- **Plasma** is obtained by adding an **anticoagulant** (such as heparin, citrate, EDTA, oxalate, or fluoride) to whole blood and then **centrifuging** it. Because plasma still contains **fibrinogen**, its total protein concentration is about **5% higher** than that of serum.
- **Serum** is the clear liquid that separates from blood after it **clots**. It is collected by allowing blood to clot and then centrifuging it to separate the liquid portion. Since **fibrinogen** is converted to fibrin during clotting, **serum contains no fibrinogen** and **no anticoagulants**.

4) Anticoagulants

Anticoagulants prevent blood from clotting, allowing collection of **whole blood or plasma**. Common anticoagulants include **EDTA, heparin, citrate, and oxalate**. Correct blood-to-anticoagulant ratio is important to avoid clots and preserve test accuracy.

a) EDTA (Ethylenediaminetetraacetic Acid)

In most clinical laboratories, **potassium EDTA** is the anticoagulant most often used for **complete blood count (CBC)** tests. This is recommended by both the **International Council of Standardization in Hematology (ICSH)** and the **Clinical and Laboratory Standards Institute (CLSI)**.

EDTA works by **binding (chelating)** calcium and other divalent ions needed for the blood-clotting process. By removing calcium, it stops the conversion of **prothrombin to thrombin**, and therefore prevents the formation of **fibrin**, which is necessary for clotting. Because of this action, **EDTA plasma should not be used for coagulation tests** such as **prothrombin time (PT)** or **activated partial thromboplastin time (aPTT)**.

EDTA helps preserve blood cell shape and count. **Hemoglobin** remains stable for about **48 hours**, and **red blood cells (RBCs)** for about **24 hours**. However, because EDTA can cause cell shrinkage over time, **blood smears should be made within 2–3 hours** after collection. **White blood cell (WBC)** counts stay stable for about **3 days** at room temperature, and platelets are also preserved, though their shape may change with time.

If there is **too little EDTA** in the tube, the blood may clot — usually due to **overfilling the tube** or **poorly dissolved EDTA (especially with disodium salts)**.

EDTA can also bind to other metals like **zinc, copper, and magnesium**, which affects the activity of metal-dependent enzymes (e.g., **alkaline phosphatase, creatine kinase**). Therefore, it is **not used for most chemistry tests** involving these enzymes.

EDTA is used for several other tests, such as **blood bank crossmatching**, **flow cytometry**, **HbA1c**, and **drug level monitoring** for immunosuppressants (e.g., **cyclosporine, tacrolimus, sirolimus, everolimus**).

When blood needs to be collected for **unstable hormones and peptides** (such as

ACTH, PTH, renin, calcitonin, cytokines, etc.), **whole blood in EDTA tubes transported on ice** is preferred.

For molecular biology work (like **DNA/RNA extraction, PCR, and sequencing**), **spray-dried potassium EDTA** is recommended because it helps maintain nucleic acid stability.

b) Heparin

Heparin is a naturally occurring anticoagulant made up of negatively charged polysaccharides. It stops clotting by activating **antithrombin**, which in turn inhibits **thrombin** and **clotting factors II (prothrombin)** and **Xa**. Because it affects these factors, **heparinized plasma is not suitable for coagulation tests**.

Heparin is commonly added to blood collection tubes (BCT) as **lithium, sodium, or ammonium salts**, usually in concentrations of **10–30 USP units per milliliter of blood**. Dry (lyophilized) heparin is preferred to avoid diluting the sample.

Heparinized plasma is ideal for **many chemistry tests**, since it doesn't bind calcium or cause water shifts in cells. It also allows for **faster testing**, as no clotting step is required and there is less risk of **microclots** interfering during pipetting.

Heparin is the **only anticoagulant recommended for blood gas and electrolyte testing**, including **pH and ionized calcium**. **Lithium heparin** is most commonly used for general chemistry; however:

- **Lithium heparin** can falsely raise lithium levels.
- **Sodium heparin** can increase sodium levels by **1–2 mmol/L**.
- **Ammonium heparin** can raise measured ammonia levels.

Heparin should **not** be used for **protein electrophoresis** or **cryoglobulin testing**, because **fibrinogen** (present in plasma) may interfere by migrating with other proteins.

Potassium Oxalate with Sodium Fluoride/Iodoacetate

Potassium oxalate acts as an anticoagulant by binding calcium, while **sodium fluoride** or **iodoacetate** stop glycolysis (the breakdown of glucose).

- **Sodium fluoride** inhibits the enzyme **enolase**.

- **Iodoacetate** inhibits **glyceraldehyde-3-phosphate dehydrogenase**.

This combination is used for measuring **glucose, lactate, and ethanol**. However, fluoride does not stop glycolysis immediately—it can take up to **4 hours**, during which **glucose may drop 5–7% per hour** at room temperature. Therefore, **fluoride tubes are not suitable for newborn (neonatal) glucose tests** unless samples are kept on ice.

c) Sodium Citrate

For coagulation studies, the **CLSI (H21-A5, 2008)** recommends using **trisodium citrate**, either **buffered or unbuffered**, in concentrations of **3.2% (preferred)** or **3.8%**. The **blood-to-anticoagulant ratio** must be **9:1**.

Citrate works by binding calcium, but its action can be **reversed by adding calcium back**, which makes it ideal for **clotting and factor assays**. It has **minimal effect on blood cells and platelets** and is also used for **platelet aggregation studies**.

Using the wrong citrate concentration can affect **PT and aPTT** results and cause variability in **INR** calculations. Therefore, labs must use the same concentration consistently.

5) Blood Collection Tubes (BCT)

Choosing the right **blood collection tube** is very important for accurate test results. Tubes come in different colors, sizes, and contain various additives such as **anticoagulants**, **clot activators**, or **separator gels**.

a) Plastic vs. Glass Tubes

Most labs now use **plastic tubes** instead of **glass** for safety reasons. Plastic tubes are **unbreakable, lighter, and disposable**, and can handle high-speed centrifugation. However, glass tubes allow better **clot adherence** and **serum separation** because of their **silica surface**.

To mimic the clotting efficiency of glass, the inside of plastic tubes is often coated with **surfactants and silicate polymers**. Still, some **minor differences** in test results can occur between glass and plastic tubes, especially in **chemistry and hematology**.

b) Stoppers and Lubricants

Tube stoppers often contain **glycerol or silicone** to make them easy to cap and remove. However, some red-top tubes may contain **trace metals (zinc, aluminum,**

magnesium), which can interfere with **heavy metal assays** or **triglyceride tests** that measure glycerol. These additives can also cause **errors in mass spectrometry**.

c) Serum Separator Gel Tubes (SST)

Separator gels form a barrier between cells and serum/plasma after centrifugation. SSTs are popular because they require only **one centrifugation step**, improve **sample stability**, and reduce the need for transferring samples to new tubes.

However, it's important to follow the **manufacturer's instructions** for **mixing, centrifugation speed, temperature, and storage**, since these factors affect gel performance and test accuracy.

6. **Order of Draw for Blood Collection Tubes**

To prevent errors in laboratory results, blood collection tubes (**BCTs**) must be filled in a specific order during **phlebotomy**. This **order of draw (OFD)** prevents carryover contamination between additives from different tubes. While laboratories may have slight variations, the general recommended order (based on **CLSI guidelines**) is:

1. **Blood culture tubes** – for microbiological testing
2. **Trace element tubes (non-additive)**
3. **Citrate tubes** – for coagulation studies
4. **Serum tubes** – with or without clot activator or gel
5. **Heparin tubes** – with or without gel
6. **EDTA tubes**
7. **Acid citrate dextrose (ACD) tubes**
8. **Glycolytic inhibitor tubes**

Tubes containing additives must be **mixed gently by inversion** according to manufacturer instructions.

- **Blood culture tubes** are filled first to prevent bacterial contamination from skin flora.
- **Trace element tubes** (usually **royal blue**) are used when testing for heavy metals; they are made to be free of metal contamination.
- **Citrate tubes** come next, as silica in plastic serum tubes can interfere with coagulation factors.
- **Serum tubes** are drawn before anticoagulant tubes to avoid contamination by anticoagulant additives.
- Among anticoagulant tubes, the order is **heparin → EDTA → glycolytic inhibitors**.

7. COLLECTION SITES; ARTERIAL, CAPILLARY, AND VENOUS BLOOD SAMPLES; COLLECTIONS FROM CATHETERS AND INTRAVENOUS LINES

Blood is the most frequently used biological specimen for diagnostic testing. The selection of collection site depends on patient age, condition, and the required investigation.

a) Venous Blood

- The **preferred sample type** for most biochemical, hematological, and serological analyses.
- Provides reliable results and is relatively easy to obtain.

Venipuncture Technique and Site Selection

Preferred Sites:

- **Median cubital vein** (most accessible and least painful).
- **Alternative veins:** Cephalic and basilic veins; dorsal hand veins if needed.

Avoid:

- Arm on the same side as a **mastectomy**
- **Scarred, bruised, or edematous areas**
- Sites above **IV lines or fistulas**

Proper site selection minimizes the risk of sample contamination and hemolysis.

b) Arterial Blood

- Primarily used for **blood gas analysis** (pH, pCO_2, pO_2).
- Commonly collected in critically ill patients or those with **respiratory disorders**.

Arterial Puncture

- Sites used (in order of preference): **Radial → Brachial → Femoral arteries**.
- Avoid inflamed or infected areas.

In **neonates**, **umbilical artery catheters** are used for arterial blood gas sampling.

c) Capillary (Skin Puncture) Blood

- Preferred in **infants and young children (<2 years)**.
- Sites:
  - **Heel** (infants)
  - **Earlobe or fingertip** (older children/adults)
- Suitable when venipuncture is difficult (e.g., in obesity, burns, thrombosis).

- Not recommended for **ESR**, **coagulation tests**, or **blood cultures**.
- Capillary blood closely resembles arterial blood for **pH** and **pCO_2**, but not for **pO_2**.

d) Indwelling Catheters and Intravenous (IV) Lines

Whenever possible, **blood collection should be avoided near an active intravenous line**. It is preferable to choose a site on the **opposite arm** or one that is **distal (below)** to the IV line.

Although blood can be collected directly through **indwelling catheters** (such as central venous or arterial lines), this method must be handled carefully to prevent **sample contamination or dilution** with IV fluids.

To reduce contamination:

- The **IV valve should be closed for at least 3 minutes** before collecting the specimen.
- To remove any IV fluid remaining in the tubing, **withdraw and discard 6–10 mL of blood** before taking the actual sample.
- If the line contains **heparin** (used to maintain patency), a **larger discard volume** may be needed, especially for **coagulation studies**, to prevent interference.
- Blood drawn from **catheters or IV lines should not be used for culture tests**, since bacteria growing in the line can lead to **false-positive results**.

Contamination Control and Site Preparation

Proper skin antisepsis before phlebotomy is essential to prevent contamination.

- **70% isopropyl alcohol** is the **preferred antiseptic** for routine blood collection.
- The site should be allowed to **air dry for 30–60 seconds** to reduce the risk of contamination and to prevent interference in **alcohol assays**.
- **Iodine-based disinfectants** (e.g., povidone-iodine) may interfere with some **biochemical tests** and are best avoided for routine chemistry collections.
    - Povidone-iodine can **falsely increase potassium, phosphorus, and uric acid** levels, particularly in samples collected by skin puncture.

- Iodine may be reserved for cases where **alcohol use is contraindicated**, such as in patients with **alcohol sensitivity or allergy**.

8.Tourniquet Application and Its Effects

Tourniquet use is essential for venous access but must be limited to **less than one minute** to avoid biochemical changes due to **venous stasis**.

- Applying a tourniquet at about **60 mmHg pressure** can lead to **anaerobic metabolism**, causing **increased lactate and ammonia** and **lowered blood pH**.
- **Tissue compression** may cause leakage of **intracellular components**, leading to falsely elevated **potassium and enzymes**.
- **Prolonged application (>3 minutes)** causes **hemoconcentration**, resulting in:
    - **8–10% increase** in enzymes, proteins, and other cell-associated substances.
    - **Documented changes** after 1–3 minutes include:
        - Total protein ↑ **4.9%**
        - Iron ↑ **6.7%**
        - Lipids ↑ **4.7%**
        - Cholesterol ↑ **5.1%**
        - AST ↑ **9.3%**
        - Bilirubin ↑ **8.4%**
        - Potassium ↓ **6.2%**

Additional factors such as **stress, hyperventilation**, and **muscle contraction** (from repeated fist clenching) can elevate **glucose, cortisol, muscle enzymes, potassium**, and **free fatty acids**. Hence, venous occlusion time should be kept as short as possible to maintain accurate test results.

9. Sample Processing and Misidentification — Major Sources of Pre-Analytical Errors

Errors during **sample handling, labeling, or processing** are major contributors to inaccurate laboratory results. Mistakes can occur from the time a specimen is collected until testing begins.

10. Effect of Centrifugation on Test Results

Some analytes in unprocessed serum or plasma are unstable. Therefore, the **Clinical and Laboratory Standards Institute (CLSI)** recommends that **plasma or serum be separated from blood cells as soon as possible**, and within **2 hours of collection**. Centrifugation is an essential part of sample processing. However, **improper**

centrifugation techniques can produce **inaccurate test results**.

Before centrifugation, samples must be **properly prepared** to ensure reliable results:

- **Serum samples** should be given enough time to **clot completely** before centrifugation.
    - Tubes **without clot activators** may require up to **60 minutes**.
    - For patients taking **anticoagulant medications**, clotting may take even longer.
- **Plasma samples** should be **gently mixed** according to the manufacturer's instructions to ensure the **anticoagulant is fully dispersed**.
    - If the sample is **not mixed well**, incomplete anticoagulant mixing can lead to **platelet clumping or clot formation**, which prevents proper separation of plasma or serum from the cells during centrifugation.

Some analytes in unprocessed serum or plasma are unstable. Therefore, the **Clinical and Laboratory Standards Institute (CLSI)** recommends that **plasma or serum be separated from blood cells as soon as possible**, and definitely **within 2 hours of collection**.

Samples should **never be opened before or during centrifugation**, as this may cause **evaporation** and alter test results.

Certain tests, such as those for **ionized calcium** and **pH**, require **anaerobic conditions** (no air exposure). If these samples are exposed to air before centrifugation, **carbon dioxide (CO_2) escapes**, leading to:

- **Increased pH**
- **Decreased ionized calcium levels**

Centrifugation is an essential part of sample processing. However, **improper centrifugation techniques** can produce **inaccurate test results**.

Before centrifugation, samples must be **properly prepared** to ensure reliable results:

- **Serum samples** should be given enough time to **clot completely** before centrifugation.
    - Tubes containing **clot activators** must be mixed properly and allowed to clot for at least **30 minutes**.

Thus, proper handling and centrifugation are critical to maintaining accurate results.

10.Transportation of Specimens

All samples must be transported from the collection area to the testing or processing site while maintaining **specimen integrity**. Transportation conditions such as **time, temperature, and turbulence** can directly affect test accuracy.

a) Transportation Time

Efforts should be made to **minimize the time delay** between blood collection and sample processing. Some analytes (test components) are **highly sensitive to time** and may change if blood remains in contact with cells for too long.

Blood cells remain metabolically active after collection, especially at **room temperature (RT)** or higher. This can alter the chemical composition of the sample.

- In **uncentrifuged whole blood** kept at RT, **glucose levels decrease by 5–7% per hour** due to ongoing glycolysis.
- Even after **centrifugation**, if serum or plasma is **not separated** from the cells, glucose continues to drop and may become clinically unreliable within **4 hours**.

According to the **Clinical Laboratory Standards Institute (CLSI)** guidelines, **plasma or serum should be separated from cells within 2 hours** of collection. Most analytes are stable for more than 2 hours, so samples received after this period do not always need to be rejected.

However, if laboratories strictly follow these standards, **centrifuges and processing stations** should ideally be available at every collection point to allow immediate sample separation.

Therefore, laboratories must develop **clear policies** to:

- Identify samples of poor quality
- Decide when to **accept or reject** such specimens

b) Effects of Temperature

Maintaining proper **temperature control** during transport is crucial to avoid pre-analytical errors. Adjusting transport temperature can help preserve analyte stability.

In general, **lower temperatures** improve the stability of most analytes, but there are **some exceptions**:

- **Cold temperatures** slow glycolysis, which reduces ATP production.
  Without ATP, cell membrane **Na^+/K^+ pumps** fail, causing potassium to leak out of cells. As a result, **potassium levels may falsely increase** if a specimen is stored in the cold for more than **6 hours**.
- If a sample is kept at **room temperature**, **glucose is consumed** by cells for glycolysis. This maintains normal potassium levels but leads to **falsely low glucose values**.

This creates a challenge when transporting samples for **basic metabolic panel testing**. The problem can be solved by **separating plasma or serum from the cells before transport**.

Some analytes degrade quickly at room temperature and **must be kept chilled**, such as:

- **Ammonia**
- **Lactate**
- **Pyruvate**
- **Parathyroid hormone-related protein (PTHrP)**
- **Gastrin**

These samples should be **chilled immediately after collection** and kept cold throughout transport and processing.

Sometimes, testing is **deliberately delayed**, such as during **batch testing** or when sending samples to **reference laboratories**.
In such cases, samples may be **frozen** to preserve stability.
However, some enzymes are **sensitive to freezing**:

- When stored in **liquid nitrogen**, the following enzymes remain stable: **AST, ALT, ALP, GGT, and LDH**.
- **Amylase activity increases**, while **creatine kinase (CK)** activity **decreases significantly** when frozen at **–20°C**, even for short durations.

c) **Humidity** is another important factor, especially for **dried blood spot (DBS)** samples exposed to air. High humidity causes faster degradation of analytes such as:

- **Biotinidase**
- **Galactose-1-phosphate uridyl transferase**
- **Glucose-6-phosphate dehydrogenase (G6PD)**
- **Thyroxine (T4)**

Humidity can also lead to **inaccurate readings** in self-monitoring blood glucose meters.

To prevent this:

- Transport samples in **sealed plastic bags** with **desiccant packets**
- Use **humidity indicator cards** to monitor moisture levels

(to be continued in next issue)

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